Molecular Biology

ANALYSIS OF *PSEUDOMONAS AERUGINOSA* POLYSACCHARIDE GENES THAT PLAY A ROLE IN BIOFILM FORMATION

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Pseudomonas aeruginosa is an opportunistic pathogen that causes chronic lung infections in cystic fibrosis (CF) patients. *P. aeruginosa* infections initially have a non-mucoid phenotype, but over time, the bacteria becomes mucoid. A mucoid phenotype is characterized by the overproduction of the exopolysaccharide alginate. The mode of growth for the bacteria in these infections is a biofilm, which allows the bacteria to be more resistant to antibiotics and the host immune system. The structure of the biofilm is believed to be supported by secreted polysaccharides, which aid the bacteria in its resistance to antibiotics as well as host defenses. Several polysaccharides, including alginate, have been suggested to play a role in the development and/or structure of the *P. aeruginosa* biofilm. This study seeks to determine how conserved the proposed polysaccharide loci are in a wide variety of *P. aeruginosa* isolates. Both mucoid and non-mucoid CF isolates as well as non-mucoid isolates from acute infections were analyzed. Primers were designed specifically for one gene from each loci. Polymerase chain reaction (PCR) was used to amplify the gene fragment from each strain and these were subsequently resolved by agarose gel electrophoresis. Of the five genes studied, which included algD, EPS1, EPS2, EPS3, and EPS4, three of the genes were contained in all 13 strains analyzed. Gene EPS2 showed some variance in the band size between the non-mucoid and two mucoid strains. One of the other three mucoid strains tested for this gene did not appear to contain it. Gene EPS3 was contained within all the strains analyzed except one mucoid strain.

The study further sought to determine if any antibiotic resistance is conferred upon the bacteria in the presence of a polysaccharide. The two polysaccharides examined were EPS2 and EPS3. Antibiotic biofilm assays were performed comparing the wild type and mutant strains of these two polysaccharides. Microtiter dish grown biofilms were exposed to varying concentrations of three antibiotics: carbenicillin, cipromycin, and tobramycin. The minimum concentration of antibiotics needed to kill all the bacteria in a biofilm (MBC) was determined for each strain and each antibiotic. Conclusive results were not found. This data suggests that these polysaccharide loci are highly conserved across many types of *P. aeruginosa* isolates, however data regarding the role of two exopolysaccharides in antibiotic resistance were not conclusive.